ANAEROBIC MICROBIAL COMPOSITION AND METHODS OF USING SAME

This application is a divisional application of U.S. Ser. No. 12/131,666 filed on Jun. 2, 2008 which claims priority of provisional patent application Ser. No. 60/936,017 filed on Jun. 13, 2007, the entireties of which are incorporated herein by reference.

I. FIELD OF THE INVENTION

The present invention relates to an anaerobic microbial composition and to methods of using the microbial composition for effectively dechlorinating at least one of chlorinated ethanes, chlorinated ethenes, chlorinated methanes, or mixtures thereof.

II. BACKGROUND OF THE INVENTION

Bioaugmentation (site inoculation with a microbial culture) is a proven approach for stimulating complete dechlorination of sites contaminated with chlorinated ethenes. However, cultures have not been available for the large-scale treatment of chlorinated ethane contamination. Of additional concern, chlorinated ethanes can inhibit the degradation of chlorinated ethenes. Thus, cultures are needed for bioremediation of sites with mixtures of these contaminants.

Contamination of groundwater with chlorinated ethenes and ethanes is a serious problem due to widespread and historic commercial, industrial, and military use, relative resistance to degradation, and associated health hazards. Under anaerobic conditions, chlorinated ethenes and ethanes can be partially reduced to less chlorinated compounds or completely degraded to nonchlorinated end products depending on the physiological capability of an indigenous microbial community.

Bacterial isolates capable of reducing 1,2-dichloroethane (DCA) and 1,1,1-trichloroethane have been identified. One isolate has been shown to reduce 1,1,2,2-tetrachloroethane (TeCA) to cis 1,2-dichloroethene (cisDCE). Recent research on a mixed culture demonstrated growth of *Dehalobacter* sp. with the reduction of 1,1,2-trichloroethane (TCA) to vinyl chloride (VC). See Jones et al., *Characterization of a Microbial Consortium Capable of Rapid and Simultaneous Dechlorination of* 1,1,2,2-*Tetrachloroethane and Chlorinated Ethane and Ethene Intermediates*, Bioremediation Journal, 10:153-168 (2006), the disclosure of which is incorporated herein by reference in its entirety.

III. SUMMARY OF INVENTION

According to an aspect of the invention, a composition is provided for concurrent dechlorination of a mixture of chlorinated ethanes and chlorinated ethenes. The composition includes an isolated bioremediative consortium comprising strains of microorganism comprising *Clostridium, Acetobacter, Dehalobacter, Bacteroides*, and Proteobacteria.

According to another aspect of the invention, a composition is provided for concurrent dechlorination of a mixture of chlorinated ethanes and chlorinated ethenes. The composition includes a non-naturally occurring consortium of dechlorinatingly effective microbial species. The consortium of effective dechlorinatingly microbial species comprises at least one 16S rDNA nucleic acid sequence that has more than 95% identity to a nucleic acid sequence consisting of SEQ ID NO 1, a nucleic acid sequence consisting of SEQ ID NO 2, or a nucleic acid sequence consisting of SEQ ID NO 3.

According to another aspect of the invention, a method for dechlorinating chlorinated waste is provided including contacting at least one of chlorinated ethanes, chlorinate ethenes, or chlorinated methanes with an isolated bioremediative consortium comprising strains of microorganism comprising Clostridiales, Cytophaga-flavobacterium-bacterioides, Proteobacteria, and Methanomicrobia; and anaerobically dechlorinating the at least one of chlorinated ethanes, chlorinate ethenes or chlorinated methanes.

According to another aspect of the invention, a method of producing a microbial consortium comprises culturing microbes of a sediment sample obtained from a site contaminated with a mixture of chlorinated ethanes and chlorinated ethenes in an anaerobic medium with at least one chlorinated ethane and an electron donor.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates possible pathways of anaerobic 1,1,2,2-tetrachloroethane (TeCA) dechlorination. Compounds for which the EPA reports an increased risk of cancer are indicated with an asterisk.

FIGS. **2**A-**2**B illustrate dechlorination in stock microbial cultures according to the present invention amended with lactate and (A) TeCA only or lactate and (B) TeCA, TCA and cisDCE.

FIG. **3** illustrates degradation of ethene and production of ethane in a microbial culture according to the present invention after depletion of chlorinated compounds.

FIGS. **4**A-**4**B illustrate cisDCE (A) and TCA (B) degradation by stock microbial cultures according to the present invention grown with (1) TeCA only and (2) a mixture of TeCA, TCA, and cisDCE, and (3) no culture added.

FIGS. **5**A-**5**B illustrate production and degradation of chlorinated intermediates of (A) TCA and (B) cisDCE in a microbial culture according to the present invention.

FIGS. **6**A-**6**B illustrate dechlorination of added compounds and accumulation of intermediates in cultures transferred to fresh medium and amended with lactate and either (A) TeCA only or (B) TeCA, TCA, and cisDCE.

FIGS. **7**A-**7**D illustrate a comparison of TRFLP profiles for APG sediments WB23 (A) and WB30 (B), and for a microbial composition according to the present invention after 1 year in culture with TeCA (C) or a mixture of chlorinated compounds (D).

FIGS. **8**A-**8**B illustrate frequency of phylogenetic types in a microbial composition rDNA clone library (A) and mcr clone library (B) according to the present invention.

V. DETAILED DESCRIPTION OF INVENTION

The present invention is directed to an anaerobic microbial composition or consortium comprising bioremediative organisms. The invention is also directed to methods of using the microbial composition for the effective dechlorination of at least one of chlorinated ethanes, chlorinated ethenes, chlorinated methanes, or mixtures thereof. The consortium of the present invention may be employed for bioremediation to anaerobically biodegrade chlorinated waste, for example, contaminated groundwater or contaminated soil from landfill sites, river beds, lakes, wetlands, and the like.

FIG. **1** illustrates that the TeCA degradation pathway is primarily biotic and includes both hydrogenolysis to less chlorinated ethanes and dichloroelimination to less chlorinated ethenes. Abiotic production includes the production of trichloroethene (TCE) from dehydrochlorination of TeCA.